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(54) Title: PEPTIDES FOR USE IN THE TREATMENT OF ALZHEIMER'S DISEASE

(57) Abstract: Anti-sense peptides that correspond to Amyloid-ß protein residues 1-43 are identified, and are used to identify protein binding sites on enzymes that interact with Amyloid-ß. The anti-sense peptides can be used as, or to identify, therapeutic agents that prevent Amyloid-ß cytotoxicity, and may be useful in the treatment of Alzheimer's disease. The anti-sense peptides show sequence similarity to the protein kinase cdc2, and it has now been found that the cytotoxic form of Aß is phosphorylated.

PEPTIDES FOR USE IN THE TREATMENT OF ALZHEIMER'S DISEASE

Field of the Invention

This invention relates to peptides and drugs that target proteins implicated in the progression of Alzheimer's disease. The peptides are also highly specific targets for therapeutic reagents that are useful for detecting, preventing and treating Alzheimer's disease.

Background of the Invention

Alzheimer's disease is a debilitating physical disease, responsible for just over half of the 670,000 cases of dementia in the UK. One of its proposed mechanisms of action is via an alteration in the structure of the Amyloid-ß (Aß) protein (Selkoe, Nature 399: A23-A31 (1999)).

The Aß protein is generated from the Amyloid-ß Precursor Protein (AßPP) with the major forms Aß 1-42 and Aß 1-40 and the N-terminally truncated P3 peptides (Aß 17-40 and Aß 17-42) being generated by alternative enzymatic processing of AβPP. The C-terminally extended forms of Aß (Aß 1-42 and Aß 17-42) show increased ability to form fibrils and are thought to have a causative action in the neurodegeneration seen in Alzheimer's disease (Mattson, Physiol. Rev. 77: 1081-1132 (1997); Rosenblum, J. Neuropath. Exp. Neurol. 58: 575-581 (1999)).

All the major forms of Aß contain a functional neurotoxic domain (Aß 25-35) and mediate their neurotoxicity by binding to the intracellular Aß-binding protein ERAB, an alcohol dehydrogenase (Yan *et al.*, J. Biol. Chem. 274: 2145-2156 (1999); Yanker *et al.*, Science 250: 279-282 (1990)). The major forms of Aß also inhibit hydrogen peroxide breakdown by the antioxidant enzyme catalase, an effect that involves a direct high affinity binding reaction (Milton, Biochem. J. 344: 293-296 (1999)).

The Aß 31-35 peptide is the shortest cytotoxic form of Aß, inhibits catalase and inhibits binding of Aß 1-42 to catalase (Milton, Biochem. J. 344: 293-296 (1999)). Both catalase and antibodies specific to this region prevent Aß cytotoxicity, suggesting that compounds which specifically bind Aß 31-35 may be of therapeutic value in the treatment of Alzheimer's disease.

The Aß 16-20 region has been shown to be responsible for binding to

ERAB (Oppermann *et al.*, FEBS Lett. 451: 238-242 (1999)). Antibodies which block Aß binding to ERAB prevent Aß 1-42 cytotoxicity, suggesting that compounds which specifically bind Aß 16-20 may also antagonise actions of Aß.

It has also been proposed that an alteration in the structure of the Aß protein may be an important determinant of cytotoxicity (Selkoe, Nature 399: A23-A31 (1999)). Chronic inhibition of phosphatases can cause Alzheimer's-like pathology (Arendt *et al.*, Neurobiol. Aging; 19:3-13 (1998)) suggesting that Alzheimer's pathology may be due to an imbalance of kinase/phosphatase levels. The appearance of Aß plaques in such animal models suggests that phosphorylation actions are crucial in the biochemical processes underlying Aß plaque formation. The ability of cyclin-dependent kinase inhibitors to prevent Aß toxicity also suggests a key role for such kinases in the toxic actions of Aß (Giovanni *et al.*, J. Biol. Chem; 274:19011-6 (1999); Alvarez *et al.*, FEBS Lett; 459: 421-6 (1999)). These enzymes specifically phosphorylate serine and threonine residues within substrates and play roles in cell division and apoptosis.

The cyclin-dependent kinase cdc2 phosphorylates the tau protein, which is a major component of the neurofibrillary tangles characteristic of Alzheimer's disease. The cdc2 kinase also phosphorylates the AßPP and this event is thought to modulate the processing events which lead to the production of the mature Aß peptide forms. There are, however no known cdc2 recognition sites on the Aß peptide itself.

Anti-sense peptide sequences are derived from the complementary strand of DNA encoding a given protein, read in the same open reading frame (ORF). They can also be derived directly from the amino acid sequence of a protein, via reverse translation to produce a complementary DNA sequence. However, due to the degeneracy of the genetic code, there is typically more than one anti-sense sequence for any one protein. The complementary DNA strand for each individual amino acid can be read in either the forward 3'-5' or reverse 5'-3' direction, adding further degeneracy to the potential anti-sense peptide sequences. Anti-sense peptides have been shown to bind with high affinity to the given protein due to hydropathic interactions. Anti-sense peptides have also been shown to have sequence similarity to receptor binding sites and

compounds, such as antibodies, that specifically bind such anti-sense peptides, have been used to isolate receptors (Bost & Blalock, Methods Enzymol; 168: 16-28 (1989), the content of which is incorporated herein by reference).

Summary of the Invention

The present invention is based on the surprising finding that Aß anti-sense peptides (AßAS) have sequence similarity with components of the cyclin-dependent kinase enzyme complex and that Aß is phosphorylated in the Alzheimer's brain. This suggests (for the first time) that there is a direct biochemical interaction between Aß and cyclin-dependent kinase enzymes.

The present invention is also based on the discovery of amino acid sequence similarities between an AßAS peptide and specific regions in ERAB and catalase.

According to a first aspect of the invention, a peptide comprises the anti-sense sequence of Aß 1-43, or a fragment thereof, capable of binding to the Aß protein within the Aß 1-43 region, or a homologue thereof with the same hydropathic profile, or at least 60% sequence similarity.

Peptides of the invention may be used to target the Aß protein to prevent phosphorylation by a protein kinase, or to prevent binding to catalase. Alternatively, the peptides may be used in assays to identify therapeutic agents that are capable of preventing interactions between Aß and a protein kinase, or which modify interactions between Aß and catalase.

According to a second aspect of the invention, the peptides may be used in the manufacture of a medicament for therapy of a condition mediated by either phosphorylation of Aß, and/or the binding of endogenous Aß to catalase.

According to a third aspect of the invention, the peptides are used in an assay for the identification of an agent that either prevents phosphorylation of Aß, and/or inhibits the binding of endogenous Aß to catalase. The assay comprises contacting a target agent with a peptide of the invention and Aß protein, and determining whether the agent prevents the peptide binding to Aß protein, when compared against a control where no target agent is present.

The realisation that cdc2 enzyme can interact with a specific region of Aß, resulting in Aß phosphorylation, allows new treatments to be developed, to

prevent phosphorylation, and to treat Alzheimer's disease.

According to fourth aspect of the invention, a protein kinase inhibitor is used in the manufacture of a medicament for the treatment of Alzheimer's disease, the inhibitor being targeted to prevent phosphorylation of the Aß protein, to exert its therapeutic effect.

The present invention may also be used in a diagnostic application.

According to a fifth aspect of the invention, a method for determining whether a patient is at risk from Alzheimer's disease, comprises analysing a patient sample that contains Aß to determine whether any of the Aß is phosphorylated, where the detection of phosphorylated Aß indicates a risk of Alzheimer's disease.

The anti-sense peptides of the invention may also be used in a vaccine. Further, a phosphorylated Aß fragment may be used, either to generate antibodies specific for the phosphorylated form, or as an antigen in a vaccine composition.

Brief Description of the Drawings

The present invention is illustrated with reference to the following figures, wherein:

Figure 1 shows the AßAS forward (F) peptide sequences derived from the cDNA strand complementary to the coding strand, i.e. read in the 3'-5' direction, "*" refers to a stop codon and "Alt AA" refers to an alternative amino acid which may be used as a replacement due to degeneracy of the sequence in the coding (5' to 3') strand;

Figure 2 shows the AßAS reverse (R) sequences, where "Rev 3'" refers to the DNA of the complementary strand, read in the 5' to 3' direction for each amino acid coding triplet;

Figure 3 shows the AßAS consensus (C) sequence derived from a comparison of AßAS(F) and AßAS(R) sequences;

Figure 4 shows a comparison of an Aß anti-sense amino acid sequence with the amino acid sequences of cyclin-dependent kinase enzymes;

Figure 5 shows the binding of biotinylated Aß 1-40 to recombinant human cdc2 in the presence of Aß fragments, the cdc2 substrate peptide CSH 103 and

the AßAS(F) peptides 14-23 and 27-36;

Figure 6 shows the phosphorylation of biotinylated Aß 1-42 (hatched columns), Aß1-40 (open columns) and Aß 25-35 (closed columns) by human cdc2/cyclin-B1 in the presence of Aß 17-28, the cdc2 119 – 122 fragment (CDK1P) and the purinergic cdc2 inhibitor olomoucine;

Figure 7 shows the effects of Aß 17-35 (open circles), Aß17-35 S26A derivative (open squares) and Aß17-35 pS26 (closed squares) on the MTT reduction in human NT-2 neurons;

Figure 8 shows the levels of phosphorylated Aß peptide measured in extracts of human NT-2 neurons after exposure to Aß 17-35 derivatives in the presence (closed columns) or absence (open columns) of the cdc2 inhibitor olomoucine;

Figure 9 shows the binding of biotinylated Aß 1-40 to recombinant human cyclin B1 in the presence of Aß fragments, the cdc2 substrate peptide CSH 103 and the AßAS(F) peptides 14-23 and 27-36;

Figure 10 shows the effects of Aß peptides and olomoucine on human cdc2/cyclin-B1 phosphorylation of the histone H1 peptide;

Figure 11 shows the effects of Aß peptides alone (open columns) or in the presence of the AßAS(F) 14-23 peptide (closed columns) or AßAS(F) 27-36 peptide (hatched columns) on the viability of SP2/0-Ag-14 mouse myeloma cells; and

Figure 12 shows the effects of Aß peptides alone (open columns) or in the presence of the AßAS(F) 14-23 peptide (closed columns) or AßAS(F) 27-36 peptide (hatched columns) on catalase enzyme activity.

Description of the Invention

The present invention is based on an analysis of anti-sense peptides derived from Aß, to identify proteins that interact with the Aß protein. Comparing the anti-sense sequences with known proteins, to identity sequence homologies, identified potential binding sites on known proteins that interact with Aß. This has resulted in the identification of the precise regions of cdc-2, Cyclin B1, ERAB and catalase that are involved in protein binding.

The term "anti-sense peptide" is used herein to define an amino acid sequence that corresponds to that derived from a DNA sequence complementary to the normal coding sequence. As is well known in the art, DNA usually exists as a duplex with one strand being the coding strand which is expressed in the 5' to 3' direction. The complementary strand is not normally expressed but acts as a template for RNA polymerase, and extends in the 3' to 5' direction. The sequence of the complementary strand can be used to derive the anti-sense peptide, either through the use of synthetic methods or by recombinant DNA technology.

The principle of anti-sense peptides is that the hydropathic character of a peptide derived from the coding strand will be opposite to that derived from the complementary strand. Therefore, even though the actual anti-sense amino acid sequence will be very different from that derived from the coding strand, there will be a relationship in respect of the hydropathic character. This is explained in Blalock and Smith, Biochem. Biophys. Res. Comm. 121(1): 203-207 (1984) and Blalock and Bost, Biochem. J., 234: 679-683 (1986), the content of each being incorporated herein by reference. Because an anti-sense peptide will, in general, have a hydropathy profile opposite to that of the corresponding sense peptide, it is expected that both will undergo protein-protein interactions.

An anti-sense peptide of the invention will correspond to that derived from the complementary strand read in the 3' to 5' direction (see SEQ ID NO. 3). Further, an anti-sense peptide may also be derived by reversing the order of each trimer (amino acid encoding) DNA sequence of the complementary strand, to encode a different amino acid (see SEQ ID NO. 5). For example, if the complementary strand (3' to 5') is:

3'-AAT GAC-5' (SEQ ID NO. 11)

then the reverse sequence for each trimer is:

3'-TAA CAG-5' (SEQ ID NO. 12)

Peptides of the invention derived in this way have similar hydropathy profiles and can bind to the Aß 1-43 region.

The sequence of an anti-sense peptide may vary due to the degeneracy of the coding strand. For example, the amino acid valine is encoded by GTG or GTT. The complementary strand will therefore be either CAC or CAA encoding histidine or glutamine, respectively. This is also shown in Figures 1 and 2 for the alternative anti-sense sequences derived from Aß 1-43.

The sequence of the complementary strand may encode a stop codon. In these circumstances, it is necessary to introduce an appropriate amino acid residue. The replacement amino acid residue will usually be derived from an alternative coding sequence for the amino acid of the coding strand. For example, if the coding strand is ATC (isoleucine), the complementary strand is a stop codon TAG. Isoleucine is also encoded by ATA, the complement of which encodes tyrosine (TAT). Therefore, tyrosine is used at the position corresponding to the stop codon. This is shown in Bost & Blalock, Methods Enzymol; 168: 16-28 (1989), the content of which is incorporated herein by reference.

For the avoidance of doubt, reference to the Aß 1-43 region means the amino acid numbering for the conventional Aß protein, shown as SEQ ID NO. 2.

Functional fragments thereof, i.e. smaller peptides that retain the ability to bind to the Aß 1-43 region, are also within the scope of the invention. The fragments will usually be at least 6 amino acids in length, typically the fragments will be at least 8 amino acids in length. In preferred embodiments, the fragments comprise the anti-sense derivatives of Aß 12-24 or Aß 31-35. In further preferred embodiments, the fragments are the anti-sense derivatives of Aß 3-30, Aß 17-35, Aß 17-24, Aß 12-28, Aß 14-35 or Aß 25-35.

The binding of Aß to itself can occur in both parallel and anti-parallel orientations (Serpell, Biochimica et Biophysica Acta 1502: 16-30 (2000)) with consequent interactions between for example two N-terminals in parallel binding or an N and a C terminus in anti-parallel binding. If binding of a peptide to an anti-sense peptide sequence were to occur in an anti-parallel orientation then the anti-sense peptide would have to be synthesized in the anti-parallel direction

with the C terminus occupying the N-terminus of the resultant peptide. Similarly an anti-parallel binding interaction between a binding protein and a peptide may be identified by comparison of the anti-sense peptide sequence in the C-terminus to N-terminus orientation with the binding protein sequence in the normal N-terminus to C-terminus orientation.

The anti-sense peptides of the invention can therefore have the given sequence in either the N-terminus to C-terminus orientation, or the C-terminus to N-terminus orientation. This is demonstrated by SEQ ID NO. 4 (N-terminal amino acid first) and SEQ ID NO. 7 (C-terminal amino acid first).

The binding of a peptide (or fragment) to the endogenous Aß 1-43 region may be determined as shown in the Examples, and in Milton, Biochem. J. 344: 293-296 (1999).

The peptides bind with a dissociation constant (Kd) of less than 50 µM, preferably less than 10 µM.

The term "homologue" is used herein in two separate contexts. The first is to refer to peptide sequences that share the same hydropathy profile as the peptides of the invention. This may be determined by analysing the peptide sequence and evaluating what alternative amino acids could be used as a replacement based on hydropathic character. Table 1 groups together those amino acids with a similar hydropathic character and which can be substituted for an amino acid specified in the anti-sense peptide sequence.

Table 1

Amino acid	Acceptable substitutions
Alanine (Ala)	Arg, Gly, Pro, Ser, Thr
Arginine (Arg)	Cys, Gly, Ser, Thr, Trp
Asparagine (Asn)	Asp, Gln, Glu,, His, Lys, Tyr
Aspartic acid (Asp)	Asn, Gln, Glu,, His, Lys, Tyr
Cysteine (Cys)	Arg, Gly, Ser, Trp
Glutamic Acid (Glu)	Asn, Asp, Gln, Lys,
Glutamine (Gln)	Asn, Asp, Glu, His, Lys, Tyr
Glycine (Gly)	Ala, Arg, Cys, Ser, Thr, Trp,
Histidine (His)	Asn, Asp, Gln, Tyr
Isoleucine (Ile)	Leu, Met, Val
Leucine (Leu)	Ile, Phe, Val
Lysine (Lys)	Asn, Asp, Gln, Glu
Methionine (Met)	Ile, Val

Phenylalanine (Phe)	Leu,
Proline (Pro)	Ala, Ser, Thr
Serine (Ser)	Ala, Arg, Cys, Gly, Pro, Thr, Trp
Threonine (Thr)	Ala, Arg, Gly, Pro, Ser
Tryptophan (Trp)	Arg, Cys, Gly, Ser
Tyrosine (Tyr)	Asn, Asp, Gln, His
Valine (Val)	Ile, Leu, Met

The term "homologue" is also used to refer to peptides that share levels of sequence identity or similarity. Levels of identity or similarity between amino acid sequences can be calculated using known methods. Publicly available computer based methods include BLASTP, BLASTN and FASTA (Atschul *et al.*, Nucleic Acids Res., 25: 3389-3402 (1997)), the BLASTX program available from NCBI, and the GAP program from Genetics Computer Group, Madison WI.

The levels of identity and similarity referred to herein are based on the use of the BLASTP program. All BLAST searches were carried out using the Standard protein-protein BLAST (blastp) on the NCBI web site (www.ncbi.nlm.nih.gov/BLAST) with the BLOSUM62 matrix and Gap Costs of 11 for Existence and 1 for Extension. The statistical significance threshold for reporting matches against database sequences (E) was reset to 100 to account for the use of short peptide sequences in the search. For BLAST comparisons between AßAS peptides and already identified Aß binding proteins, the same parameters were used except that the E value was reset to 100000 to ensure identification of all potential regions of similarity. Alignments of AßAS fragments of <5 amino acids were considered non-significant under these conditions. Sequences containing significant gaps (> 10%) were not used since hydropathic binding interactions require a direct alignment of each Aß residue with its complementary anti-sense peptide or binding domain residue.

It is preferable if there is at least 60% sequence identity or similarity to the specified peptides, preferably 70%, more preferably 80% and most preferably greater than 90%, e.g. at least 95%. The peptides should retain the ability to bind to the Aß protein.

Synthetic amino acid derivatives may also be used. For example, the shifting of substitutents within an amino acid residue, from a C atom to a N atom,

to produce a peptide having greater resistance to proteolysis, and other modifications, are known and are included within the scope of this invention.

Peptides of the invention may be synthesised using conventional methods known in the art and can be obtained to order from commercial sources. Peptide synthesis methods are also disclosed in Chan & White, Fmoc Solid Phase Peptide Synthesis: A Practical Approach (2000).

Alternatively, the peptides may be produced using recombinant DNA technology that ensures that the anti-sense (complementary) DNA sequence is expressed. This can be accomplished using techniques known to those skilled in the art. For example, the DNA sequence to be expressed can be inserted into an appropriate expression vector that contains the necessary regulatory apparatus, e.g. promoters, enhancers etc, to enable expression to occur. The DNA sequence will be in the 5' to 3' direction, for expression, but will have the same nucleotide sequence as that given for the complementary (3' to 5') strand. The DNA sequence may therefore be a synthetic polynucleotide. The expression vector can then be inserted into an appropriate host cell, to enable expression to occur. Suitable methods are disclosed in Sambrook *et al*, Molecular Cloning, A Laboratory Manual (1989), and Ausubel *et al*, Current Protocols in Molecular Biology (1995), John Wiley & Sons, Inc., the content of each being incorporated herein by reference.

As stated previously, the present invention is based on the finding that the Aß protein, within the region 1-43, contains the sites that interact with other proteins, and that these protein-protein interactions may be implicated in Alzheimer's disease. The DNA sequence that encodes the Aß 1-43 region is shown as SEQ ID NO. 1 with the encoded amino acid sequence shown as SEQ ID NO. 2. The complementary DNA sequence is also shown (SEQ ID NO. 3). The anti-sense sequence is shown as SEQ ID NO. 4. The reverse anti-sense DNA sequence is shown as SEQ ID NO. 5, with its encoded product shown as SEQ ID NO. 6.

The peptide in the C-terminus to N-terminus orientation to that of SEQ ID NO. 4, is shown as SEQ ID NO. 7, and that in the C-terminus to N-terminus orientation to that of SEQ ID NO. 6 is shown as SEQ ID NO. 8.

A further AßAS sequence (SEQ ID NO. 9) was derived by use of the consensus amino acid sequences that are found at the same position of each form of anti-sense peptide, i.e. those in the forward or reverse orientation etc. This is most clearly shown in Figure 3, where a sequence alignment of the various forms of the anti-sense peptides shows which amino acids are common to each position (AßAS(C)). This is also explained in Bost & Blalock, Methods Enzymol; 168: 16-28 (1989). The peptide in the C-terminus to N-terminus orientation to that of SEQ ID NO. 9, is shown as SEQ ID NO. 10.

The investigations disclosed herein demonstrate that Aß binds to and is phosphorylated by the human cdc2 protein kinase. The cdc2 kinase is a member of the cyclin-dependent kinase (CDK) family and the structural features of CDK substrates have been characterised. These features include the presence of ß-turn regions containing the target serine or threonine residue. The serine 26 residue in Aß is located within a ß-turn region and this structural feature may be important for the Aß phosphorylation reaction. The Aß sequence, however, does not contain the cdc2 substrate consensus sequence and it is therefore likely that the enzyme-substrate complex formation between Aß and cdc2 is mediated via a novel mechanism. Anti-sense peptides are known to bind peptides via hydropathic interactions and such binding between cdc2 and Aß with an alignment of the active site of cdc2 with Aß serine 26 provides a mechanism for the observed Aß phosphorylation reaction. Since the CDK family of kinases share similar structural features around the ATP binding and phosphate group transfer residues, it is possible that Aß could be phosphorylated by other CDK kinases, and this may explain why different groups have shown roles for different CDK enzymes in Aß cytotoxicity. The sequence similarities of CDK family members around the cdc2 active site is illustrated in Figure 4.

Therefore, molecules which specifically prevent the phosphorylation of Aß may be of therapeutic use. Anti-sense peptides of at least 6 amino acids, either alone or chemically linked to other protein kinase inhibitor molecules, derived from the Aß 1-43 sequence, may act as inhibitors of Aß phosphorylation and be useful in the treatment of Alzheimer's disease.

Fragments of Aß may also be useful as antagonists, to inhibit the phosphorylation of the endogenous Aß. In this embodiment, it is desirable to administer fragments that are capable of preventing phosphorylation, but which are also non-cytotoxic. It may therefore be desirable to administer fragments that do not contain the cytotoxic portion 31-35, or which are modified at one or more of these amino acid sites.

Compounds that bind specifically to phosphorylated Aß may also be useful in the diagnosis of Alzheimer's disease. Novel antibodies may be raised using known antibody production techniques. For example, a peptide of the present invention, acting as an antigen, may be administered to an animal to produce an antibody-rich serum. This "antiserum" can be purified, to remove unwanted antibody molecules, by, for example, affinity fractionation using phosphorylated Aß. Monoclonal antibodies may also be raised by, for example, animal or *in vitro* immunisation techniques and fusion of antigen-exposed spleen cells to a myeloma cell line to produce hybridoma cell lines that secrete antibody. By screening hybridoma cell lines with a peptide of the invention, specific antibody-producing cell lines may be established.

In a preferred embodiment, a peptide fragment of the natural Aß protein in the phosphorylated state, is used to raise antibodies that are specific for phosphorylated Aß, and not for the non-phosphorylated form. The techniques of phage display or ribosome display, both of which are conventional in the art, may be used to select those antibodies with high affinity, preferably greater than 10^{-3} M, more preferably greater than 10^{-5} or 10^{-6} M. The antibodies may be useful in therapy or diagnostic assays.

A previous study has shown that immunisation with Aß prevents Alzheimer's-like pathology in an animal model (Schenk *et al.*, Nature; 400: 173-177 (1999)). The use of a phosphorylated Aß derivative may direct the body's immune system against a more cytotoxic form of Aß and hence may be a more suitable immunogen for such treatment. Thus, immunization with a phosphorylated Aß fragment may be used as a treatment for Alzheimer's disease and as a preventative medicine.

It may also be desirable to administer an antigenic fragment of the protein kinase, e.g. cdc2, or cyclin which may also act as a preventative medicine. Antibodies raised against the protein kinase or cyclin may also be of therapeutic or diagnostic use. It is preferable to use at least that part of the protein kinase or cyclin that contains the region associated with Aβ binding, as the antigenic fragment.

The immunogen may be administered via any suitable route, preferably intravenously. Suitable pharmaceutically-acceptable diluents and carriers will be known to those skilled in the art. Adjuvants may also be administered, e.g. Alum, as is known in the art. A suitable amount of the therapeutic to be administered, can be arrived at by the skilled person based on conventional formulation technology.

If the natural Aß protein (or fragments thereof), is to be used as an antigenic component of a vaccine composition, either in the phosphorylated state or non-phosphorylated state, it is desirable to ensure that the 31-35 region is deleted or modified to ensure that the Aß antigen is not cytotoxic.

In a further embodiment, if a Aß peptide is to be administered as an antigen in the non-phosphorylated form, it may be desirable to modify the peptide to replace the amino acid residue susceptible to phosphorylation, to ensure that no phosphorylation occurs.

Peptides, antibodies and compositions of the present invention may be useful in a method of treating or diagnosing Alzheimer's disease.

For example, a sample from a patient (blood sample, tissue sample etc.) that contains Aß can be used to detect whether phosphorylated Aß is present. The phosphorylated Aß can be detected, for example, by the use of an antibody that has specificity for phosphorylated Aß and no or reduced specificity for non-phosphorylated Aß. Alternatively, levels of phosphorylated Aß can be determined by measuring the cytotoxicity of the Aß sample, compared to a non-phosphorylated Aß sample.

The peptides of the invention may be used in assays to identify therapeutic molecules that can prevent phosphorylation of Aß from occurring. For example, combinatorial chemistry could be used to develop target

therapeutic molecules, which are then screened for activity. The target molecules can be brought into contact with Aß protein, or a fragment thereof comprising the phosphorylation site of Aß, and a protein kinase, e.g. p34-cdc2. If the presence of the target molecule results in reduced phosphorylation, then it may be a potential therapeutic candidate. Alternatively, the target molecule can be brought into contact with Aß protein and an anti-sense peptide of the invention, and the efficacy of the target molecule determined on the basis of a reduction in binding affinity between the Aß protein and the anti-sense peptide.

Preferably, the target molecule will be a protein kinase inhibitor that acts specifically at the Aß target site. It is therefore preferable for the target molecule to have affinity for Aß. Alternatively, a protein kinase inhibitor could be adapted to include a targeting molecule that has affinity for Aß.

In an alternative embodiment, it may be useful to identify compounds that phosphorylate Aß. Assays to identify phosphorylating compounds can be designed so that Aß (or a suitable fragment thereof) is brought into contact with the compound to be tested, in the presence of suitable reagents necessary to allow a phosphorylation reaction to proceed. The extent (if any) of phosphorylation can then be determined.

In a further embodiment, the phosphorylated Aβ may be used as a target for compounds that inhibit or modify the biological action of the phosphorylated Aβ. Assays can be carried out to determine whether a target compound interacts selectively with the phosphorylated form of Aβ and alters the Aβ cytotoxicity.

In addition, the peptides of the invention can prevent Aß binding to catalase, and may be useful in therapy or in assays to identify agents that prevent binding of Aß to catalase.

The invention will now be further described by the following Examples with reference to the accompanying Figures.

Example 1

To identify potential Aß binding domains within human proteins, an anti-sense peptide approach was used. The forward Aß anti-sense peptide (AßAS(F)) 1-43 (SEQ ID NO. 4) was derived by reading the complementary (non-coding) strand of DNA from the region encoding the Aß 1-43 peptide in the

3'-5' direction; where the DNA encoded a stop codon, the nearest suitable replacement amino acid was substituted. The AßAS(F) sequence was used in a BLAST search to identify proteins with sequence similarity. Results showed a region of sequence similarity with the AßAS(F) 3-30 sequence having 46% identity and 68% similarity with the human cdc2 105-132 region (SEQ ID NO. 15). This indicated that Aß 1-43 may be phosphorylated by cdc-2. The BLAST comparison between AßAS and human cdc2 (Accession No. GI 87058) also identified three other regions of sequence similarity. Cdc2 residues 56 to 63 (SEQ ID NO. 13) showing 50% identity and 75% similarity with AßAS 20-27; cdc2 residues 95 to 99 (SEQ ID NO. 14) showing 80% identity with AßAS 37-41; and cdc2 residues 229 to 238 (SEQ ID NO. 16) showing 40% identity and 50% similarity with AßAS 33-42.

Experiments were carried out to determine whether Aß 1-43 binds to and is phosphorylated by cdc-2. It was found that Aß 1-43 binds to and is phosphorylated by cdc-2 and that phosphorylation follows similar kinetics to known p34-cdc2 substrates and can be inhibited by chemicals and peptides known to inhibit p34-cdc2 kinases.

Biotinylated Aß 1-42, Aß 1-40 and Aß 25-35 were prepared using a LinKit-Biolink kit (ISL, Paignton, UK). ELISA plates were coated with recombinant human cdc2 or the cdc2 119-133 peptide fragment (CDKP1) (1 μg ml^{-1}) in carbonate buffer and unoccupied sites blocked with 5% (w/v) dried milk. Biotinylated peptides (200 pM) were incubated alone, with control peptides (somatostatin) or with unlabelled Aß peptides in PBS (containing 0.1% BSA and 0.05% Tween-20) at 37°C for 4 hours. After washing to remove unbound material, an alkaline phosphatase polymer-streptavidin conjugate (Sigma, Dorset, UK) was added and incubated at 37°C for 2 hours. After washing to remove unbound material p-nitrophenylphosphate substrate was added and absorbance at 405 nm determined. Affinity constants were determined by incubating cdc2 coated plates with biotinylated peptides (200 pM) plus Aß peptides over a range of concentrations (0 - 10 μM) and detection of bound peptides was carried out by ELISA.

The results showed that Aß 1-42, Aß 1-40 and Aß 25-35 bound to human

cdc2 (Figure 5). The cdc2 substrate peptide CSH-103 (Sigma) and peptides containing Aß residues 17-28 could inhibit the binding of Aß to cdc2. The AßAS(F) 14-23 but not the AßAS(F) 27-36 peptide also inhibited binding. The binding of Aß 1-40 was concentration dependent and showed an affinity constant (K_D) of 12.7 ± 4.3 µM. The biotinylated Aß peptides also bound specifically to a synthetic peptide (CDKP1) corresponding to cdc2 residues 119-133. Binding of Aß 25-35 to the CDKP1 peptide could be inhibited by peptides containing the Aß 17-28 sequence and by either anti-Aß 17-28 or anti-CDKP1 antibodies. The binding of Aß to cdc2 was inhibited by the Aß 17-28 but not the Aß 31-35 fragments indicating that the cdc2 56-63 may also contribute to Aß binding. The alignment within this cdc2 region of Aß residue 23 (a negatively charged Aspartic acid residue) with the positively charged Arginine 59 of cdc2 suggested that a charge-based interaction may occur at this location. The tertiary structure of cdc2 suggests that the 56-63 region could play a role in interactions with the substrate bound to the active site region surrounding cdc2 residue 128. These observations suggest that this Aß binding region may be an alternative target for therapeutic agents that would specifically disrupt Aß phosphorylation.

Using the NetPhos 2.0 computer program, which predicts phosphorylation sites in proteins (Blom *et al.*, J. Mol. Biol. 294: 1351-1362 (1999)) it was found that Aß 1-42 contains three potential sites (serine 8, tyrosine 10 and serine 26). The NetPhos 2.0 scores were obtained from the output score of the ensemble of neural networks trained on that acceptor residue type and a value > 0.5 was considered significant. The scores for Aß serine 8, tyrosine 10 and serine 26 were 0.963, 0.870 and 0.787 respectively. The alignment of the AßAS 26 residue, which is complementary to the Aß serine 26 residue, with the proposed aspartic acid 128 active site residue of cdc2 that is involved in the transfer of the phosphate group from ATP to the substrate (Figure 4) suggests that cdc2 could phosphorylate Aß.

For p34-cdc2 activity measurements, recombinant p34-cdc2/cyclin B1 (Promega, UK) was used. The activity of p34-cdc2 incubated with biotinylated Aß 1-42, 1-40 and 25-35 was determined. Recombinant p34-cdc2 with an activity of 1U (incorporation of 1 pmol ATP/min/µg protein into a peptide substrate of the

histone H1 sequence PKTPKKAKKL (SEQ ID NO. 22) was incubated with 25 µM biotinylated peptides in assay buffer (50 mM TRIS-HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 40 mM ß-glycerophosphate, 20 mM p-nitrophenylphosphate, 0.1 mM sodium vanadate, 50 µM ATP pH 7.4) plus test substances and [γ^{32}P]-ATP (specific activity 3,000 Ci/mmol) in a final volume of 25 µl. After incubation for 10 min at 30°C termination buffer (12.5 µl; 7.5 M guanidine.HCl) was added. A 15 µl aliquot of each sample was spotted onto a streptavidin membrane (Promega, UK) to isolate the biotinylated peptides. The membrane was washed four times in 2 M NaCl, followed by four times in 2 M NaCl containing 1% (v/v) H_3PO_4 and finally twice in deionized H_2O to remove unbound material. The radioactivity of [γ^{32}]-ATP incorporated into the biotinylated peptides was measured by scintillation counting and the enzyme activity determined.

Results showed that Aß 1-42, Aß 1-40 and Aß 25-35 incorporated ^{32}P from γ^{32}P-ATP (Figure 6) and that cdc2 caused the appearance of phosphorylated serine residues in Aß 1-42, Aß 1-40 and Aß 25-35. Phosphorylation of Aß was inhibited by olomoucine, a purinergic cdc2 inhibitor, the CDKP1 peptide, Aß 12-28 and Aß 17-28. Kinetic analysis of the reaction showed that the phosphorylation was concentration dependent and the Michaelis constant (K_M) for the phosphorylation of Aß 1-40 was 5.2 µM, which compared with a K_M of 2.7 µM for the H1 peptide substrate.

In order to assess the effects of Aß phosphorylation on the cytotoxic properties of Aß peptides, a series of Aß 17-35 derivatives were synthesised. The 17-35 region of Aß contains the serine residue (serine 26) which is proposed to be phosphorylated by p34-cdc2, and also contains the ERAB binding (17-20) and cytotoxic domains (31-35) thought to play roles in Aß cytotoxicity. The peptides were tested in a cytotoxicity assay as follows. Human NT-2 (NTera2/D1) precursor cells were propagated in DMEM/F12 medium supplemented with retinoic acid for 5-6 weeks prior to harvesting and replating in the presence of mitotic inhibitors, to generate post-mitotic Human NT-2 neurons. For cytotoxicity experiments, 5×10^3 cells/100 µl medium were plated in Poly-D-lysine coated 96 well plates. Test peptides (20 µM) were added directly to culture medium prior to incubation for 24 h. Cell viability was

determined by measurement of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction (Shearman, Methods Enzymol. 309: 716-723 (1999)). After incubation with peptides MTT (10 µl: 12mM stock) was added and cells incubated for a further 4 hours. Cell lysis buffer [100 µl/well; 20% (v/v) SDS, 50 % (v/v) N,N-dimethylformamide, pH 4.7] was added and, after repeated pipetting to lyse cells, colorimetric determination of MTT formazan product formation was determined by measuring the absorbance change at 540 nm. Control levels in the absence of peptide were taken as 100%, with the absorbance in the absence of cells taken as 0%.

The Aß 17-35 peptide caused a dose dependent reduction in MTT utilisation (Figure 7). The Aß 17-35 S26A mutated peptide, in which the serine residue for phosphorylation has been mutated to an alanine residue, had no effect on MTT utilisation indicating that this mutation abolishes the cytotoxic potential of the peptide. An Aß 17-35 peptide with a phosphorylated serine residue (Aß 17-35 (pS26)) caused a dose dependent reduction in MTT utilisation and was significantly more potent than the non-phosphorylated peptide. These results suggest that the serine phosphorylation by p34-cdc2 or other kinases could be a key step in the cytotoxic actions of Aß peptides.

To establish whether Aß is phosphorylated in the Alzheimer's brain, Alzheimer disease brain sections were obtained from Novagen Inc (Madison, WI, USA. Cat No: 70298-3; Lot No: A301036). Sections were deparaffinised and extracted in DEA buffer supplemented with 0.1mM sodium vanadate. Extracts from NT-2 neurons were also prepared using the same buffer. Using a polyclonal anti-Aß 15-30 antiserum plus Protein-A agarose, the Aß was immunoprecipitated. The resultant extracts were further purified using a Sep-Pak C_{18} extraction step. Columns were pre-wetted with methanol and 0.5M acetic acid and the samples were applied in 20% acetonitrile in 0.1% TFA. The columns were washed with 20% acetonitrile in 0.1% TFA prior to elution of bound peptide with 70% acetonitrile. After drying under a stream of nitrogen, samples were resuspended in appropriate buffer. For SDS-PAGE analysis, samples were resuspended in gel loading buffer and run on a 15% acrylamide gel. After immunoblotting onto nitrocellulose membranes, the blots were stained

with anti-Aß, anti-phosphoserine, anti-phosphotyrosine or anti-phosphothreonine antibodies. Bands were visualized with anti-mouse or anti-rabbit IgG-HRP conjugates and TMB membrane substrate.

SDS-PAGE analysis showed the presence of a phosphoserine containing band of a similar size to Aß which could also be stained with a specific anti-Aß monoclonal antibody (6F3D). The staining of this band with an anti-phosphoserine antibody, but not the anti-Aß antibody, was prevented by pretreatment of the extracts with alkaline phosphatase. No bands were stained using anti-phosphothreonine and anti-phosphotyrosine specific antibodies, confirming that the Aß was only phosphorylated at one of its serine residues.

A specific immunoassay was used to measure Aß phosphorylated on a serine residue (pSAß) in cell extracts. ELISA plates were coated with anti-phosphoserine antiserum for pSAß determination and blocked with 5% dried milk. Samples or synthetic Aß standards (Aß 17-35 pS) were applied in PBS containing 0.1% BSA plus 0.05% Tween 20. Monoclonal antibody ALI-01 was added and incubated for 2 hrs. After washing to remove unbound material, ir-pSAß was detected using an anti-rabbit IgG-HRP conjugate and TMB substrate. A similar assay in which the pS antibody was replaced with a polyclonal anti-Aß antibody was used to measure Aß levels.

Extracts from NT-2 neurons contained 3.16 ± 0.48 nmol/g Aß of which 1.30 ± 0.05 nmol/g (41.1 ± 1.6%) was of the pSAß form. Alzheimer's disease brain extracts contained 59.8 ± 3.8 nmol/g Aß of which 12.6 ± 6.6 nmol/g (20.8 ± 10.7%) was of the pSAß form. Human NT-2 neurons exposed to the Aß 17-35, Aß 17-35 pS26 and an S26A mutated Aß 17-35 derivative showed increased levels of immunoreactive Aβ (ir-Aß). Measurement of ir-pSAß in the same cell extracts showed that cells exposed to Aß 17-35 contained increased amounts of ir-pSAß (Figure 8), whilst cells exposed to the Aß 17-35 pS26 or Aß 17-35 S26A peptides showed no difference to control cells. The increase in ir-pSAß levels, but not ir-Aß, when cells were treated with Aß was prevented by the cdc2 inhibitor olomoucine.

Cyclins are co-factors for cdc2 which are required for enzyme activity. These proteins also contain substrate recognition sequences which may play a

role in the recruitment of substrate molecules to the active cdc2/cyclin complex. The recombinant cdc2/cyclin enzyme complex used in the above phosphorylation experiments contained cyclin B1. To test if this protein contained an Aß binding site the AßAS (R) reverse peptide sequence, read in the C to N direction (SEQ ID NO. 8) was used in a BLAST comparison with the cyclin B1 (GI 116176) protein sequence. Results showed a region with 30% sequence identity and 43% sequence similarity between this AßAS peptide and the cyclin B1 257-285 (SEQ ID NO. 17) region. Binding assays as described above using cyclin B1 coated plates were carried out.

Results showed that cyclin B1 bound to biotinylated Aß 1-40 and 25-35 (Figure 9). The binding was inhibited by Aß 31-35 containing peptides. The affinity constant for Aß 1-40 binding to cyclin B1 was 2.3 ± 0.5 µM. The binding could be inhibited by the forward AßAS(F) 27-36 but not the AßAS(F) 14-23 peptide.

Since Aß binds to both the cdc2 and cyclin B1 components of the active enzyme it is possible that Aß modulates the activity of the kinase. This was tested by performing kinase activity measurements using a biotinylated Histone H1 substrate peptide (PKTPKKAKKL) and measurement of incorporation of ^{32}P from ^{32}P-ATP as above. Results showed that Aß 1-40, 17-35, 25-35 and 31-35 all increased the phosphorylation of the H1 peptide by cdc2/cyclin B1 (Figure 10), suggesting that Aß could activate the kinase. The fragments capable of activation were the same as those which inhibited Aß 1-40 binding to cyclin B1 and these results suggest that the binding to cyclin B1 may be a mechanism for the enzyme activation.

Example 2

This Example shows the protein-protein interaction between the peptides of the invention and utility of the peptides of the invention as inhibitors of binding between Aß and catalase.

The BLAST comparison between the AßAS(F) 1-43 peptide (SEQ ID 4) and human catalase (GI: 14763736) identified three regions of sequence similarity. Catalase residues 402 to 414 (SEQ ID NO. 20) showing 46% identity and 61% similarity with AßAS 29-40; catalase residues 158 to 164 (SEQ ID NO.

18) showing 57% identity and 71% similarity with AßAS 25-31; and catalase residues 281 to 287 (SEQ ID NO. 19) showing 57% identity and 85% similarity with AßAS 11-17. The Aß 31-35 fragment of Aß binds to catalase (Milton 1999) and this suggests that the 402-414 region of catalase may contain the binding site. The presence of a gap in the alignment corresponding to catalase 407 which was inserted between Aß residues 33 and 34 suggests that the 402-406 region of catalase may be of more importance. This is in agreement with the study of Milton *et al.*, NeuroReport: 12, 2561-2566 (2001) which identified these residues as the binding site.

The BLAST comparison between AßAS(F) 1-43 peptide (SEQ ID NO. 4) and human ERAB (GI: 2492759) identified a single region of sequence similarity. ERAB residues 101 to 109 (SEQ ID NO. 21) showing 44% identity and 55% similarity with AßAS 16-24. The Aß 16-20 fragment of Aß binds to ERAB (Oppermann, *et al.*, FEBS Lett. 451: 238-242 (1999)) suggesting that the 101-109 region contains the ERAB binding site. This is in agreement with the proposals of Milton *et al.*, NeuroReport 12: 2561-2566 (2001) which suggested that ERAB 102-105 was the Aß binding domain.

A further comparison of the Aß peptide sequence with catalase and ERAB anti-sense sequences showed the presence of Aß-like sequences within the catalase 400-409 and ERAB 99-108 anti-sense sequences.

Synthetic peptides containing catalase residues 400-409 (CAßBP), ERAB residues 99-108 (EAßBP), AßAS(F) residues 14-23 and AßAS(F) residues 27-36 were all synthesised for analysis. The CAßBP and EAßBP peptides were tested for ability to bind biotinylated Aß. All peptides were also tested in catalase inhibition and cytotoxicity assays.

Biotinylated Aß 1-42, Aß 12-28 and Aß 25-35 (from SEQ ID NO. 2) were prepared using a LinKit-Biolink kit (ISL, Paignton, UK). ELISA plates were coated with CAßBP (catalase residues 400-409) or EAßBD (ERAB residues 99-108) (1 $\mu g\ ml^{-1}$) in carbonate buffer and unoccupied sites blocked with 5% (w/v) dried milk. Biotinylated peptides (200 pM) were incubated alone, with control peptides or with unlabelled Aß peptides in PBS (containing 0.1% BSA and 0.05% Tween-20) at 37°C for 4 hours. After washing to remove unbound material, an alkaline phosphatase polymer-streptavidin conjugate (Sigma, Dorset, UK) was added and

incubated at 37°C for 2 hours. After washing to remove unbound material, p-nitrophenylphosphate substrate was added and absorbance at 405 nm determined. Affinity constants were determined by incubating catalase or CAßBP coated plates with biotinylated peptides (200 pM) plus Aß peptides over a range of concentrations (0 - 100 nM) and detection of bound peptides was carried out by ELISA.

SP2/0-Ag-14 mouse myeloma cells were maintained in RPMI 1640 medium containing 10% fetal calf serum at 37°C in a 5% CO_2 humidified atmosphere. For cytotoxicity experiments 2 x 10^5 cells were plated in 24 well dishes in 1 ml PBS containing 0.1% BSA and test peptides (20 µM) for 24 hours. Cell viability was determined by trypan blue dye exclusion with at least 100 cells counted per well (Milton, Biochem. J. 344: 293-296 (1999)).

For catalase activity catalase EC 1.11.1.6 from human erythrocytes (Sigma, Dorset, UK) was used for all incubation experiments. Activity of Catalase (5kU l^{-1}) incubated with test peptides (2 µM) was determined after incubation in 60 mM sodium-potassium phosphate buffer at 37°C in a total volume of 100 µl. After incubation catalase activity was determined by mixing 50 µl sample with 50 µl substrate (6.5 µmol H_2O_2 in phosphate buffer) for 60 secs, adding 100 µl of 32.4 mM ammonium molybdate and measurement of absorbance change at 405 nm. Catalase activity was calculated from a standard curve (0-100kU l^{-1}) using purified human catalase of known activity (Milton, Biochem. J. 344: 293-296 (1999)).

The CAßBP peptide specifically bound biotinylated Aß 1-42 and Aß 25-35 but not Aß 12-28. Binding of the CAßBP peptide to biotinylated Aß 1-42 was inhibited by fragments of Aß 1-42 containing residues 31-35. Scatchard analysis of Aß 1-42 binding was carried out according to Friguet *et al*, J. Imm. Meth.; 77: 305-319 (1985). Data demonstrated a K_D = 1.2 ± 0.1 nM (n=5) for Aß 1-42 binding the CAßBP peptide. The binding specificity of the CAßBP peptide is identical to that for catalase and the binding K_D for catalase is comparable at 3.3 nM (Milton, Biochem. J. 344: 293-296 (1999)).

The CAßBP peptide was able to block the inhibition of catalase enzyme activity by Aß 1-42 and Aß fragments containing residues 31-35. The CAßBP

peptide was also able to block the cytotoxicity of Aß 1-42 and Aß fragments containing residues 31-35.

The CAßBP and AßAS(F) 27-36 (from SEQ ID NO. 4) sequences show sequence similarity. The AßAS(F) 27-36 peptide was also able to block the cytotoxicity of Aβ 1-42 and Aβ fragments containing residues 25-35 (Figure 11). The AßAS(F) 27-36 peptide was also able to block the inhibition of catalase enzyme activity by Aß 1-42 and Aß fragments containing residues 25-35 (Figure 12).

The EAßBP peptide specifically bound biotinylated Aß 1-42 and Aß 12-28 but not Aß 25-35. Binding of the EAßBP peptide to biotinylated Aß 1-42 was inhibited by fragments of Aß 1-42 containing residues 17-24. Scatchard analysis of Aß 1-42 binding data demonstrated a K_D = 107 ± 21 nM (n=5) for Aß 1-42 binding the EAßBD peptide. The binding specificity of the EAßBD peptide is similar to that for ERAB and the binding K_D for ERAB is comparable at 88.3 nM (Oppermann, *et al.*, FEBS Lett. 451: 238-242 (1999); Yan, *et al.*, Nature 389: 689-695 (1997)).

The EAßBP peptide had no effect on the inhibition of catalase enzyme activity by Aß 1-42. The EAßBP peptide was able to block the cytotoxicity of Aß 1-42 and Aß fragments containing residues 17-35, but not the cytotoxicity of Aß 25-35, in agreement with a binding specificity for Aß 17-24.

The EAßBP and AßAS(F) 14-23 sequences show sequence similarity. Like EAßBP, the AßAS(F) 14-23 peptide was able to block the cytotoxicity of Aß 1-42 and Aß fragments containing residues 17-35, but not the cytotoxicity of Aß 25-35 (Figure 11). The AβAS(F) 14-23 peptide had no effect on the inhibition of catalase enzyme activity by Aβ 1-42 and Aβ fragments (Figure 12).

Accordingly, peptides which can bind to the Aß protein sequence within the Aß 1-42 region, preferably the Aß 17-35 region, will be of use. Suitable peptides may be derived from the anti-sense peptides identified herein.

CLAIMS

1. A peptide comprising the anti-sense sequence of Aß 1-43 (SEQ ID NO. 2), or a fragment thereof capable of binding to the Aß protein within the Aß 1-43 region, or a homologue of the peptide or the fragment having the same hydropathic profile or at least 60% sequence identity.

2. A peptide according to claim 1, wherein the fragment comprises the anti-sense sequence of Aß 17-24 or Aß 31-35.

3. A peptide according to claim 1 or claim 2, which consists of the anti-sense sequence of Aß 3-30, Aß 17-35, Aß 12-24, Aß 12-28, Aß 14-35 or Aß 25-35, or a homologue thereof with at least 60% sequence identity.

4. A peptide according to any preceding claim, comprising any of the sequences identified herein as SEQ ID NOS. 2, 6, 7, 8, 9 or 10 or a fragment thereof capable of binding to the Aß protein within the Aß 1-43 region.

5. A peptide according to any preceding claim, having a therapeutic or diagnostic agent bound thereto.

6. A peptide according to claim 5, wherein the diagnostic agent is a detectable label.

7. A peptide according to claim 5, wherein the therapeutic agent is an inhibitor of a protein kinase.

8. A peptide according to any preceding claim for use in therapy.

9. A phosphorylated Aβ protein, or a fragment thereof, for use in therapy.

10. A protein according to claim 9, comprising a phosphorylated serine 26 residue.

11. An isolated recombinant vector comprising a polynucleotide that encodes a peptide according to any of claims 1 to 4.

12. An antibody, raised against a peptide according to any of claims 1 to 4.

13. An antibody, raised against a protein according to claim 9 or claim 10, the antibody having no or reduced affinity for the non-phosphorylated form of the protein.

14. An antibody raised against a peptide comprising any of the sequences defined herein as SEQ ID NOS. 13 to 21.

15. An antibody according to claim 14, raised against a peptide comprising any of the sequences defined herein as SEQ ID NOS. 13 to 17.

16. An antibody raised against a peptide comprising any of the sequences defined herein as SEQ ID NOS. 13 to 16.

17. Use of a peptide according to any of claims 1 to 4, in the manufacture of a medicament, for therapy of a condition mediated by phosphorylation of Aß.

18. Use of a peptide comprising the amino acid sequence Aß 1-43, or a fragment thereof capable of binding to cyclin-dependent kinase, in the manufacture of a medicament for therapy of a condition mediated by phosphorylation of Aß.

19. Use of a peptide according to any of claims 1 to 4, in the manufacture of a medicament for therapy of a condition mediated by the binding of endogenous Aß to catalase.

20. Use according to any of claims 17 to 19, wherein the condition is Alzheimer's disease.

21. Use of a protein kinase inhibitor in the manufacture of a medicament for the treatment of Alzheimer's disease.

22. Use according to claim 21, wherein the inhibitor selectively binds to Aß protein.

23. Use according to claim 21 or claim 22, wherein the kinase is p34-cdc2.

24. A method for determining whether a patient is at risk from Alzheimer's disease, comprising analysing a sample from the patient that contains Aβ to determine whether Aß is phosphorylated, where the detection of phosphorylation indicates a risk of Alzheimer's disease.

25. A method according to claim 24, wherein phosphorylation is to be detected within the Aß 1-43 region.

26. A method according to claim 24 or claim 25, wherein the phosphorylation to be detected is the phosphorylation of a serine amino acid residue.

27. A method according to any of claims 24 to 26, wherein the sample is treated with an antibody that has affinity for Aß phosphorylated within the Aß 1-43 region, and has no or reduced affinity for non-phosphorylated Aß.

28. An assay for the identification of an agent that inhibits the interaction of Aβ protein with other proteins, comprising contacting Aβ protein or a fragment thereof, with a target agent and a peptide that binds to Aβ (or the fragment), and determining whether the agent inhibits the peptide from binding to Aβ, compared to a control assay carried out in the absence of the peptide.

29. An assay according to claim 28, wherein Aβ comprises at least Aβ 1-43.

30. An assay according to claim 28 or claim 29, wherein the peptide is that according to any of claims 1 to 4, a protein kinase enzyme, or cyclin, or a fragment thereof.

31. An assay according to claim 30, wherein the protein kinase is cdc2.

32. An assay according to claim 28 or claim 29, wherein the peptide is catalase or ERAB, or a fragment thereof.

33. An assay according to claim 32, wherein the peptide is less than 40 amino acids in length and comprises any of the sequences defined herein as SEQ ID NOS. 18 to 21.

34. An assay for the identification of an agent that binds to Aß within the region Aß 1-43, comprising contacting a target agent with a peptide, as defined in claim 18, and determining whether the agent binds to the peptide.

35. An assay according to claim 34, wherein the peptide is phosphorylated.

36. A vaccine composition, comprising a peptide according to any of claims 1 to 4, and a pharmaceutically acceptable diluent or adjuvant.

37. A vaccine composition, comprising a phosphorylated Aβ protein, or a fragment thereof, and a pharmaceutically acceptable diluent or adjuvant.

38. A vaccine according to claim 36 or claim 37, comprising a Aß peptide phosphorylated on one or more of residues 8, 10, 26 and 43, and a pharmaceutically acceptable diluent.

39. A vaccine composition, comprising a peptide comprising any of the sequences defined herein as SEQ ID NO. 13 to SEQ ID NO. 17.

40. A compound that blocks the activity of phosphorylated Aβ protein.

Figure 1

Amyloid-ß (Aß) forward anti-sense peptide – AßAS(F)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aß	D	A	E	F	R	H	D	S	G	Y	E	V	H	H	Q
DNA 5'	GAT	GCA	GAA	TTC	CGA	CAT	GAC	TCA	GGA	TAT	GAA	GTT	CAT	CAT	CAA
DNA 3'	CTA	CGT	CTT	AAG	GCT	GTA	CTG	AGT	CCT	ATA	CTT	CAA	GTA	GTA	GTT
AßAS(F)	L	R	L	K	A	V	L	S	P	I	L	Q	V	V	V
Alt AA					S			R		M		H			

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Aß	K	L	V	F	F	A	E	D	V	G	S	N	K	G	A
DNA 5'	AAA	TTG	GTG	TTC	TTT	GCA	GAA	GAT	GTG	GGT	TCA	AAC	AAA	GGT	GCA
DNA 3'	TTT	AAC	CAC	AAG	AAA	CGT	CTT	CTA	CAC	CCA	AGT	TTG	TTT	CCA	CGT
AßAS(F)	F	N	H	K	K	R	L	L	H	P	S	L	F	P	R
Alt AA		D	Q						Q		R				
		E													

	31	32	33	34	35	36	37	38	39	40	41	42	43
Aß	I	I	G	L	M	V	G	G	V	V	I	A	T
DNA 5'	ATC	ATT	GGA	CTC	ATG	GTG	GGC	GGT	GTT	GTC	ATA	GCG	ACA
DNA 3'	TAG	TAA	CCT	GAG	TAC	CAC	CCG	CCA	CAA	CAG	TAT	CGC	TGT
AßAS(F)	*	*	P	E	Y	H	P	P	Q	Q	Y	R	C
Alt AA	Y	Y		N		Q			H	H			W
				D									

Figure 2

Amyloid-ß (Aß) reverse anti-sense peptide – AßAS(R)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aß	D	A	E	F	R	H	D	S	G	Y	E	V	H	H	Q
DNA 5'	GAT	GCA	GAA	TTC	CGA	CAT	GAC	TCA	GGA	TAT	GAA	GTT	CAT	CAT	CAA
DNA 3'	CTA	CGT	CTT	AAG	GCT	GTA	CTG	AGT	CCT	ATA	CTT	CAA	GTA	GTA	GTT
Rev 3'	ATC	TGC	TTC	GAA	TCG	ATG	GTC	TGA	TCC	ATA	TTC	AAC	ATG	ATG	TTG
AßAS(R)	I	C	F	E	S	M	V	*	S	I	F	N	M	M	L
Alt AA	V	R G S	L	K	A P T	V	I	A R G T	A P T	V	L	D H Y	V	V	

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Aß	K	L	V	F	F	A	E	D	V	G	S	N	K	G	A
DNA 5'	AAA	TTG	GTG	TTC	TTT	GCA	GAA	GAT	GTG	GGT	TCA	AAC	AAA	GGT	GCA
DNA 3'	TTT	AAC	CAC	AAG	AAA	CGT	CTT	CTA	CAC	CCA	AGT	TTG	TTT	CCA	CGT
Rev 3'	TTT	CAA	CAC	GAA	AAA	TGC	TTC	ATC	CAC	ACC	TGA	GTT	TTT	ACC	TGC
AßAS(R)	F	Q	H	E	K	C	F	I	H	T	*	V	F	T	C
Alt AA	L	E K	N D Y	K	E	R G S	L	V	N D Y	A P S	A R G T	I	L	A P S	R G S

	31	32	33	34	35	36	37	38	39	40	41	42	43
Aß	I	I	G	L	M	V	G	G	V	V	I	A	T
DNA 5'	ATC	ATT	GGA	CTC	ATG	GTG	GGC	GGT	GTT	GTC	ATA	GCG	ACA
DNA 3'	TAG	TAA	CCT	GAG	TAC	CAC	CCG	CCA	CAA	CAG	TAT	CGC	TGT
Rev 3'	GAT	AAT	TCC	GAG	CAT	CAC	GCC	ACC	AAC	GAC	TAT	CGC	TGT
AßAS(R)	D	N	S	E	H	H	A	T	N	D	Y	R	C
Alt AA	N Y	D Y	A P T	Q K		N D Y	P S T	A P S	D H Y	N H Y	N D	C G S	R G S

Figure 3

Amyloid-ß (Aß) anti-sense consensus peptide – AßAS(C)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aß	D	A	E	F	R	H	D	S	G	Y	E	V	H	H	Q
AßAS(F) Alt AA	L	R	L	K	A S	V	L	S R	P	I M	L	Q H	V	V	V
AßAS(R) Alt AA	I V	C R G S	F L	E K	S A P T	M V	V I	* A R G T	S A P T	I V	F L	N D H Y	M V	M V	L
AßAS(C)	X	R	L	K	X	V	X	R	P	I	L	H	V	V	X

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Aß	K	L	V	F	F	A	E	D	V	G	S	N	K	G	A
AßAS(F) Alt AA	F	N D E	H Q	K	K	R	L	L	H Q	P	S R	L	F	P	R
AßAS(R) Alt AA	F L	Q E K	H N D Y	E K	K E	C R G S	F L	I V	H N D Y	T A P S	* A R G T	V I	F L	T A P S	C R G S
ASAß(C)	F	E	H	K	K	R	L	X	H	P	R	X	F	P	R

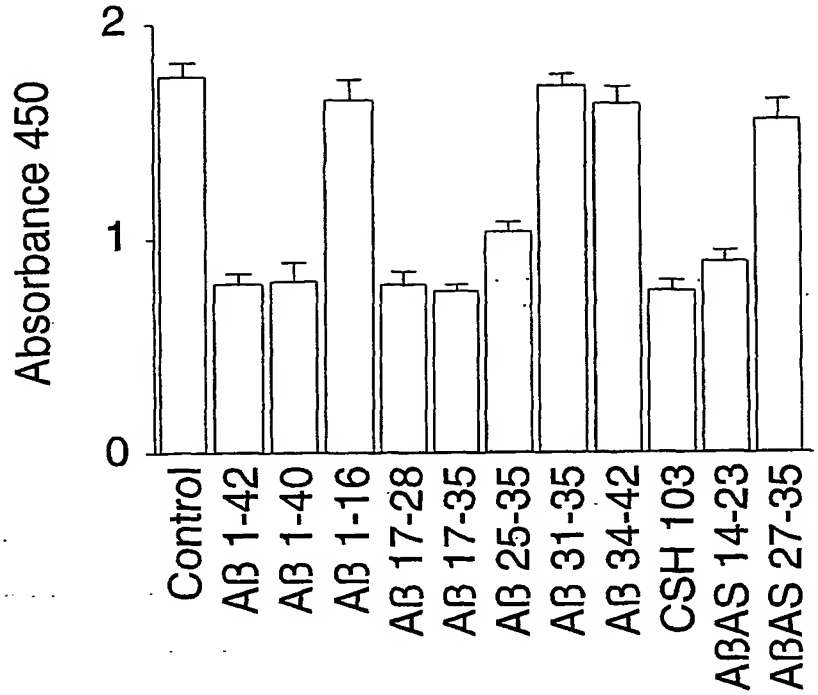
	31	32	33	34	35	36	37	38	39	40	41	42	43
Aß	I	I	G	L	M	V	G	G	V	V	I	A	T
AßAS(F) Alt AA	* Y	* Y	P	E N D	Y	H Q	P	P	Q H	Q H	Y	R	C W
AßAS(R) Alt AA	D N Y	N D Y	S A P T	E Q K	H	H N D Y	A P S T	T A P S	N D H Y	D N H Y	Y N D	R C G S	C R G S
AßAS(C)	Y	Y	P	E	X	H	P	P	H	H	Y	R	C

Figure 4

Comparison of Amyloid-ß (Aß), AßAS(F) and cyclin-dependent kinase family members amino acid sequences

Aß (3 - 30)	E F R H D S G Y E V H H Q K L V F F A E D V G S N K G A
AßAS(F) (3 - 30)	L K A V L S P I L Q V V V F N H K K R L L H P S L F P R
hCdc2 (105 - 132)	V K S Y L Y Q I L Q G I V F C H S R R V L H R D L K P Q
CDK2 (104 - 131)	I K S Y L F Q L L Q G L A F C H S H R V L H R D L K P Q
CDK3 (104 - 131)	I K S Y L F Q L L Q G V S F C H S H R V I H R D L K P Q
CDK4 (117 - 144)	I K D L M R Q F L R G L D F L H A N C I V H R D L K P E
CDK5 (103 - 130)	V K S F L F Q L L K G L G F C H S R N V L H R D L K P Q
CDK6 (122 - 149)	I K D M M F Q L L R G L D F L H S H R V V H R D L K P Q
CDK7 (114 - 141)	I K A Y M L M T L Q G L E Y L H Q H W I L H R D L K P N
CDK8 (128 - 155)	V K S L L Y Q I L D G I H Y L H A N W V L H R D L K P A
CDK9 (126 - 153)	I K R V M Q M L L N G L Y Y I H R N K I L H R D M K A A
CDK10 (140 - 167)	V K C I V L Q V L R G L Q Y L H R N F I I H R D L K V S
PCTK1 (263 - 290)	V K L F L F Q L L R G L A Y C H R Q K V L H R D L K P Q
PCTK2 (290 - 317)	V K L F L Y Q I L R G L A Y C H R R K V L H R D L K P Q
PCTK3 (148 - 175)	V K I F M F Q L L R G L A Y C H T R K I L H R D L K P Q
KKIALRE (104 - 131)	V K S I T W Q T L Q A V N F C H K H N C I H R D V K P E
CDC2L (200 - 227)	I K S F M R Q L M E G L D Y C H K K N F L H R D I K C S

Figure 5



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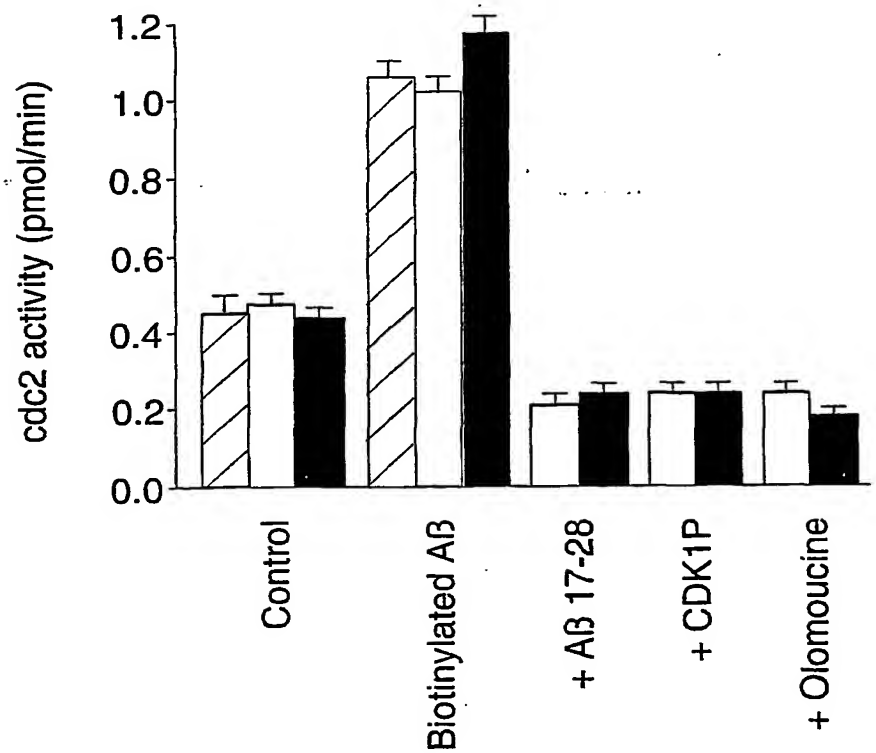

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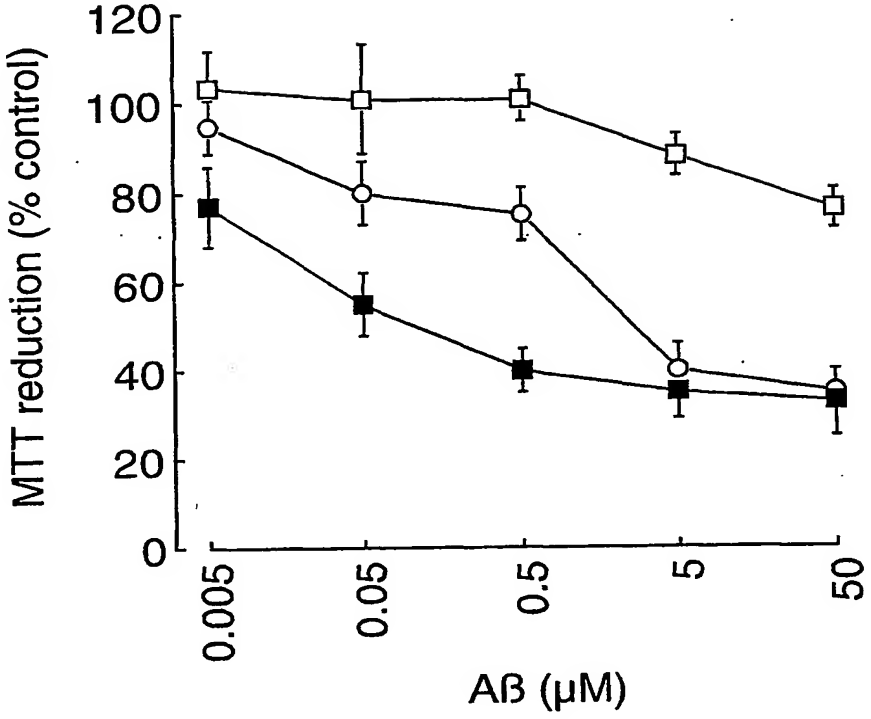

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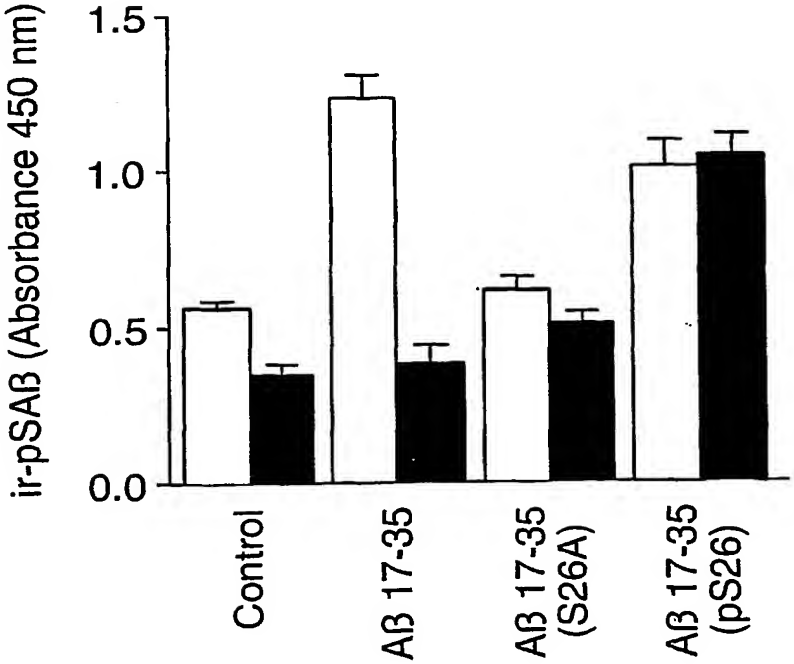

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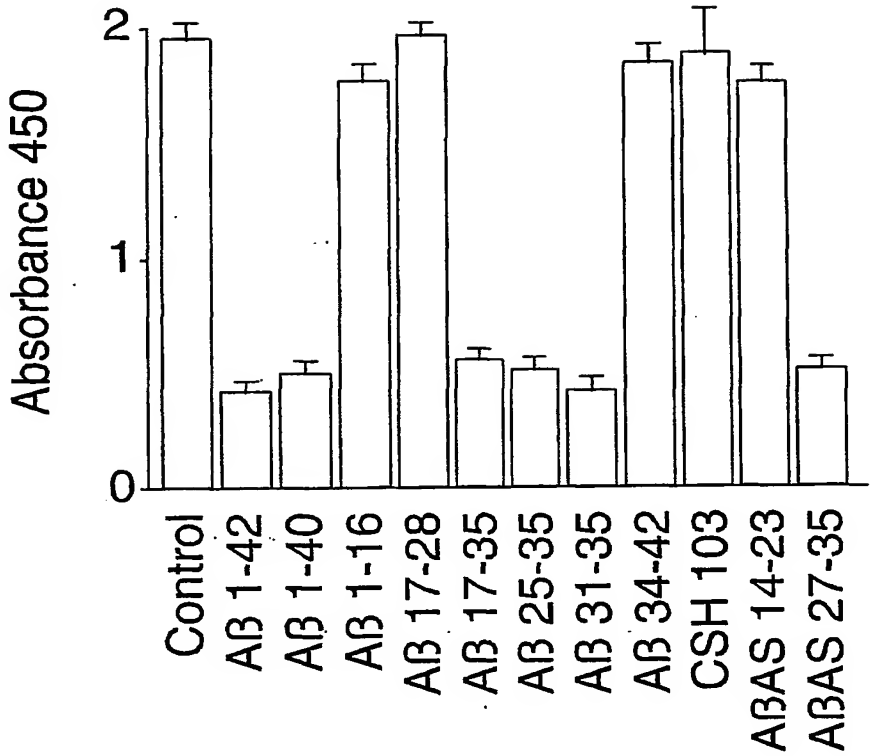

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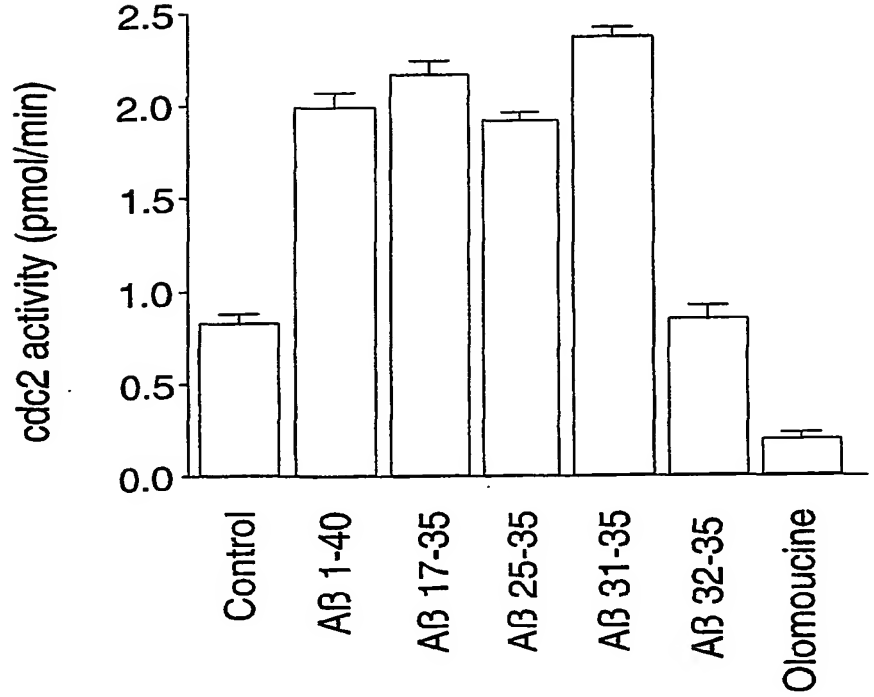

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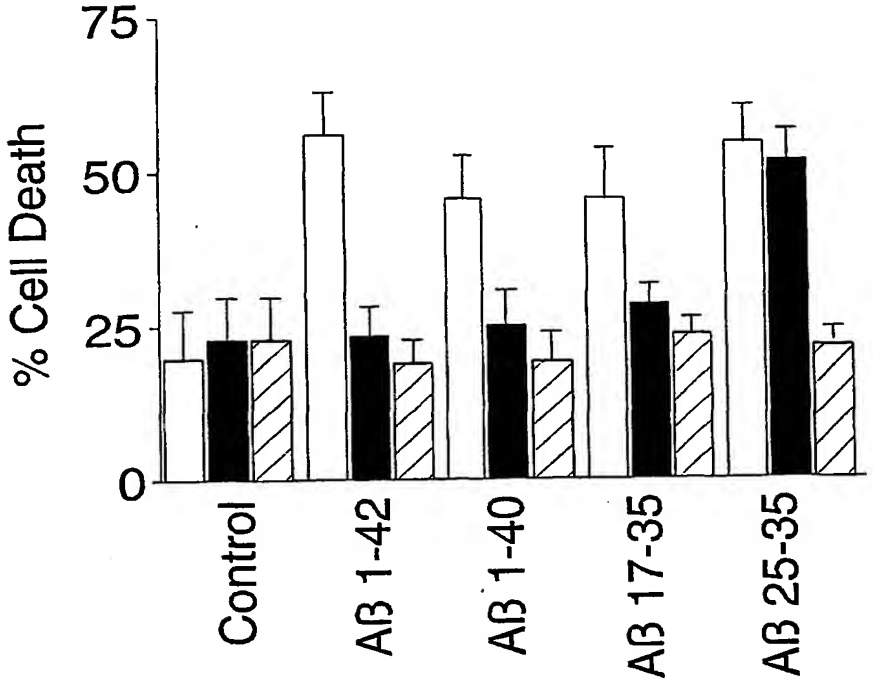

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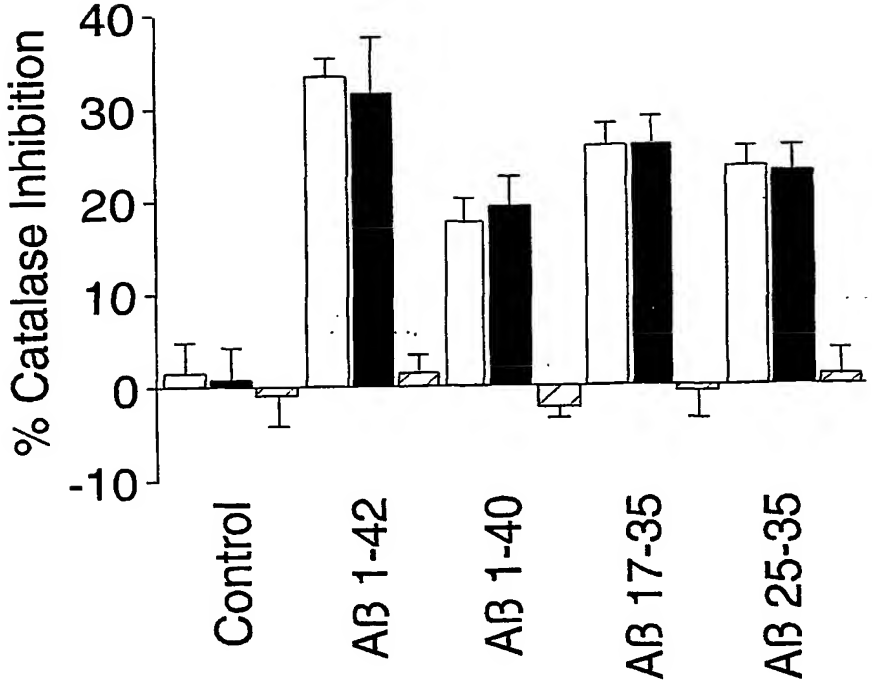

SEQUENCE LISTING

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Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
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(71) Applicant *(for all designated States except US)*: **INSIGHT BIOTECHNOLOGY LIMITED** [GB/GB]; P O Box 520, Wembley, Middlesex HA9 7YN (GB).

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(74) Agent: **GILL JENNINGS & EVERY**; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
2 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/036614 A3

(54) Title: PEPTIDES FOR USE IN THE TREATMENT OF ALZHEIMER'S DISEASE

(57) Abstract: Anti-sense peptides that correspond to Amyloid-ß protein residues 1-43 are identified, and are used to identify protein binding sites on enzymes that interact with Amyloid-ß. The anti-sense peptides can be used as, or to identify, therapeutic agents that prevent Amyloid-ß cytotoxicity, and may be useful in the treatment of Alzheimer's disease. The anti-sense peptides show sequence similarity to the protein kinase cdc2, and it has now been found that the cytotoxic form of Aß is phosphorylated.

INTERNATIONAL SEARCH REPORT

Internal application No
PCT/GB 01/04843

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 C12N15/63 C07K16/18 A61K38/17 A61K38/57
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 1 January 1998 (1998-01-01) TAYLOR C B ET AL: "Identification and characterization of genes with unstable transcripts (GUTs) in tobacco" Database accession no. 024158 XP002211379 *75% identity with SEQ ID NO:4 in 12 aa overlap (21-32:39-50)* ---	1
X Y	WO 94 15967 A (PREDDIE RICK E ;BERGMANN JOHANNA E (DE)) 21 July 1994 (1994-07-21) *abstract, page 14, line 5-page 29 line 31 and claims* --- -/--	1,8,11, 12 1-8,11, 12,17, 19,20, 30,36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 November 2002	09.01.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	BULCAO DE MELO .., T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat application No
PCT/GB 01/04843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 837 449 A (ECKER DAVID J ET AL) 17 November 1998 (1998-11-17) *abstract, column 2, line 49- column 13, line 52 and claims*	1-8,11, 12,17, 19,20, 30,36
X,P	MILTON NATHANIEL G N ET AL: "Identification of amyloid-beta binding sites using an antisense peptide approach." NEUROREPORT, vol. 12, no. 11, 2001, pages 2561-2566, XP002211378 ISSN: 0959-4965 cited in the application *whole document: in particular figures 1-3*	1-8,11, 12,17, 19,20, 30,36
A	EP 0 586 790 A (GEN HOSPITAL CORP ;MASSACHUSETTS INST TECHNOLOGY (US)) 16 March 1994 (1994-03-16) *abstract and figures 1-4*	
A	US 5 854 204 A (KELLEY MICHAEL ET AL) 29 December 1998 (1998-12-29) *abstract and * *abstract, table IV, SEQ ID NOS:1 and 2 and claims*	
X	US 5 385 915 A (BUXBAUM JOSEPH D ET AL) 31 January 1995 (1995-01-31) *whole document, in particular: abstract and claims*	9,10,13, 17,18, 24-27, 35,37, 38,40
X	SUZUKI TOSHIHARU ET AL: "Phosphorylation of Alzheimer beta-amyloid precursor-like proteins." BIOCHEMISTRY, vol. 36, no. 15, 1997, pages 4643-4649, XP002223097 ISSN: 0006-2960 *whole document, in particular: abstract and discussion*	9,10,13, 17,18, 24-27, 35,37, 38,40

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INTERNATIONAL SEARCH REPORT

Internati plication No
PCT/GB 01/04843

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IIJIMA K ET AL: "Neuron-specific phosphorylation of Alzheimer's beta-amyloid precursor protein by cyclin-dependent kinase 5." JOURNAL OF NEUROCHEMISTRY. UNITED STATES SEP 2000, vol. 75, no. 3, September 2000 (2000-09), pages 1085-1091, XP002223098 ISSN: 0022-3042 *whole document, in particular: abstract and discussion* ---	9,10,13, 17,18, 24-27, 35,37, 38,40
X	SUZUKI T ET AL: "CELL CYCLE-DEPENDENT REGULATION OF THE PHOSPHORYLATION AND METABOLISM OF THE ALZHEIMER AMYLOID PRECURSOR" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 13, no. 5, 1994, pages 1114-1122, XP002942431 ISSN: 0261-4189 *whole document, in particular: abstract and discussion* ---	9,10,13, 17,18, 24-27, 35,37, 38,40
X	WALTER JOCHEN ET AL: "Ectodomain phosphorylation of beta-amyloid precursor protein at two distinct cellular locations." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 3, 1997, pages 1896-1903, XP002223099 ISSN: 0021-9258 *whole document, in particular: abstract and discussion* -----	9,10,13, 17,18, 24-27, 35,37, 38,40

INTERNATIONAL SEARCH REPORT

Inte ial application No.
PCT/GB 01/04843

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 40
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-13, 17-20, 24-27, 30, 35-38, 40

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 40

Present claim 40 relates to a compound which is not clearly characterised by technical features, but only by the result to be achieved. Claim 40 therefore relates to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is not found in the present application. Therefore, in the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search for the subject-matter of claim 40 not possible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3 (completely) and 4-8, 11, 12, 17, 19, 20, 30 and 36 (partially)

Invention 1 concerns an antisense peptide of amyloid-beta 1-43. Invention 1 further concerns a vector comprising a polynucleotide encoding said peptide, an antibody against said peptide, a vaccine comprising said peptide as well as the use of said peptide in and assay and in the manufacture of a medicament.

2. Claims: 28, 29, 31, 32 and 34 (completely) and 4-8, 11, 12, 17-20, 22, 24-27, 30, 33 and 36 (partially)

Invention 2 concerns the amyloid-beta 1-43 peptide. Invention 2 further concerns a vector comprising a polynucleotide encoding said peptide, an antibody against said peptide, a vaccine comprising said peptide as well as the use of said peptide in several assays and in the manufacture of a medicament.

3. Claims: 9, 10, 13, 35, 37, 38 and 40 (completely) and 17, 18 and 24-27 (partially)

Invention 3 concerns a phosphorylated amyloid-beta protein. Invention 3 further concerns an antibody against said peptide, vaccines comprising said peptide as well as the use of said peptide in several assays and in the manufacture of a medicament.

4. Claims: 14-16 and 39 (completely) and 33 (partially)

Invention 4 concerns antibodies against a peptide comprising any of the sequences SEQ ID NO: 13-21 (residues from cdc2, cyclin B, catalase and ERAB proteins), an assay using a peptide comprising any of the sequences SEQ ID NO: 18-21 (residues from catalase and ERAB proteins) and a vaccine comprising a peptide comprising any of the sequences SEQ ID NO: 13-17 (residues from cdc2 and cyclin B proteins).

5. Claim : 21 and 23 (completely) and 22 (partially)

Invention 5 concerns the use of a protein kinase inhibitor in the manufacture of a medicament.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/GB 01/04843

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